

WO 00/53797

PCT/EP00/02011

Attachment to the filing of February 9, 2001

What is Claimed is

Claims:

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1. Use of a method comprising at least the following steps:
  - a) providing a cell culture container with an interior space and an inside wall and with a first and second membrane system located in the interior space, whereby a cell culture space is formed between the membrane systems and the inside wall of the interior chamber;
  - b) providing cells as a cell culture and a cell culture medium in the cell culture chamber;
  - c) adding a fluid nutrient medium to the cell culture chamber and removing metabolic products from the cell culture chamber by means of the first membrane system;
  - d) adding at least one gaseous medium to the cell culture chamber by means of a second membrane system;
  - e) metering at least one active substance into the cell culture chamber, with the metering taking place according to an adjusted active substance concentration-time curve; and
  - f) monitoring cell vitality.
- for in-vitro testing of active substances in cells.
2. Use according to Claim 1, characterized in that cytostatics, antibiotics, cytokines, growth factors, or antiviral agents are used as active substances.

3. Use according to one or more of Claims 1 or 2, characterized in that primary cells are added as the cell culture.

4. Use according to one or more of Claims 1 or 2, characterized in that tumor cell lines are used as the cell culture.

5. Use according to one or more of Claims 1 to 4, characterized in that the cell culture chamber has a volume of at least 0.1 ml minimum and 5 ml maximum.

6. Use according to Claim 5, characterized in that the cell culture chamber has a minimum volume of 0.3 ml and a maximum volume of 3.0 ml.

7. Use according to one or more of Claims 1 to 6, characterized in that at least one semipermeable membrane or at least one hydrophilic microporous membrane is used as the first membrane system and at least one gas transfer membrane is used as the second membrane system.

8. Use according to one or more of Claims 1 to 7, characterized in that the first and the second membrane systems consist of hollow fibers stacked in multiple layers.

9. Use according to one or more of Claims 1 to 8, characterized in that a cell culture container is used which has a removable lid and allows the cell culture to be prepared by adjusting the desired cell density in the cell culture medium, opening the lid of the cell culture container, pipetting the desired volume of cell suspension into the cell culture container, and closing the cell culture container using the lid.

10. Use according to one or more of Claims 1 to 9, characterized in the fact that RPMI 1640 is used as the cell culture medium.

11. Use according to one or more of Claims 1 to 10, characterized in that at least  $1 \cdot 10^5$  cells per ml of cell culture space are used.

12. Use according to one or more of Claims 1 to 11, characterized in that each cell is at an average distance of 0  $\mu\text{m}$  to 600  $\mu\text{m}$  from the closest membrane in the first and second membrane systems.

13. Use according to one or more of Claims 1 to 12, characterized in that a fluid nutrient medium RPMI 1640 is used.

14. Use according to one or more of Claims 1 to 13, characterized in that the gaseous medium has a  $\text{pO}_2$  of 0 to 160 mmHg and a  $\text{pCO}_2$  of 0 to 115 mmHg.

15. Use according to one or more of Claims 1 to 14, characterized in that the cell culture medium contains a bicarbonate buffer and the  $\text{pCO}_2$  in the gaseous medium added is adjusted so that the pH value of the cell culture medium is between 6.8 and 7.8.

16. Use according to one or more of Claims 1 to 15, characterized in that gaseous metabolic products are removed from the cell culture space by means of the second membrane system.

17. Use according to one or more of Claims 1 to 16, characterized in that individual active substances and/or combinations of several active substances are added on a time-staggered basis.

18. Use according to one or several of Claims 1 to 17, characterized in that the active substance dosage is added to the cell culture chamber directly or by means of the first membrane system.

19. Use according to one or more of Claims 1 to 18, characterized in that specification of the active substance concentration-time curve takes place with the permeabilities of the first membrane system, by the duration of the active substance administration, and by the active substance concentration.

20. Use according to one or more of Claims 1 to 19, characterized in that the cell culture container is kept at 37°C.

21. Use according to one or more of Claims 1 to 18, characterized in that the cell vitality is monitored by means of a cell vitality dye.

22. Use according to Claim 21, characterized in that Alamar Blue® serves as a cell vitality dye.

23. Use according to one or more of Claims 1 to 22, characterized in the fact the cell vitality is monitored using at least one sensor.

24. Use according to Claim 23, characterized in that a fluorescence sensor is used.

25. Device for in-vitro testing of active substances in cells, comprising a cell culture container (1) suitable for collecting a cell culture in a cell culture medium with an internal chamber (2), with first means for supplying at least one nutrient medium and second means for adding at least one gaseous medium located in the interior space, with the means each having a supply side and a removal side, and with a cell culture space being formed between said means and the inside wall of the interior chamber, and with the first means in a fluid connection with the supply side connected by nutrient medium dispensing unit (3) with at least one nutrient medium container (4), and the second means connected in a fluid connection with the supply side connected by a gas metering unit (5) with at least one gas supply container (6), characterized in that the cell culture chamber has a volume of at most 5 ml and at least 0.1 ml, and that the device also contains means (7), (8), (9a), (9b), and (9c) for supplying at least one active substance to the cell culture chamber and means for creating an active substance concentration-time curve in the cell culture chamber.

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26. Device according to Claim 25, characterized in that the first means is in a fluid connection on the removal side with a waste container (10).

27. Device according to Claim 25, characterized in that the first means is in a fluid connection on the removal side by a recirculation line (11) with the at least one nutrient medium container (4).

28. Device according to one or more of Claims 25 to 27, characterized in that the first means consists of at least one fluid medium suitable for administration.

29. Device according to one or more of Claims 25 to 28, characterized in that the second means consists of at least one membrane suitable for gas exchange.

30. Device according to one or more of Claims 25 to 29, characterized in that cell culture container (1) has a bottom and a lid which bound the interior chamber, are opposite one another, and consist of transparent material.

31. Device according to Claim 30, characterized in that a heating system is integrated into the bottom of cell culture container (1).

32. Device according to one or more of Claims 25 to 31, characterized in that the at least one membrane of the first means is a semipermeable membrane or a hydrophilic microporous membrane.

33. Device according to one or more of Claims 25 to 32, characterized in that the at least one membrane of the second means is an oxygenation membrane.

34. Device according to one or more of Claims 25 to 33, characterized in that the membranes of the first and second means are hollow fibers.

35. Device according to one or more of Claims 25 to 34, characterized in that the hollow fibers are stacked in several layers in the interior chamber.

36. Device according to Claim 35, characterized in that the maximum distance between the hollow fibers forming each means is between 50 μm and 600 μm.

37. Device according to one or more of Claims 25 to 36, characterized in that the cell culture chamber has a volume of 0.3 ml to 3.0 ml.

38. Device according to one or more of Claims 25 to 37, characterized in that the means for adding the active substance consist of at least one active substance supply container (7), at least one active substance metering device (8), and a system of lines (9) which connects the at least one active substance supply container (7) through an active substance metering unit (8) directly (9a) or through first means (9b) with the cell culture chamber of cell culture container (1).

39. Device according to one or more of Claims 25 to 38, characterized in that the device has a means for monitoring cell vitality.

40. Device according to Claim 39, characterized in that the means for monitoring cell vitality consists of at least one sensor.

41. Device according to Claim 40, characterized in that the sensor is a fluorescence sensor.

42. Modular active substance testing system comprising at least two devices according to Claims 25 to 41.

43. Modular active substance testing system according to Claim 42, consisting of 6, 24, or 96 devices according to Claims 25 to 41.

44. Use of the device according to one or more of Claims 25 to 41 or of the modular active substance testing system according to one of Claims 42 or 43 for in-vitro testing of the effects of active substances on cells.

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45. Use of the device or of the modular system according to Claim 44,  
characterized in that the influence of pharmacokinetics on cell vitality is determined.